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Shigeko Yoshida* Rriko Akagi†

Toshihiko Ubuka‡

*Okayama University,

†Okayama University,

‡Okayama University,

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Shigeko Yoshida, Rriko Akagi, and Toshihiko Ubuka

Abstract

Excretion of sulfate and taurine, two major metabolites of sulfur, was examined in rats to study the nutritional status of sulfur metabolism in the mammals. Rats maintained on a conventional laboratory diet excreted 1.83 +/- 0.14 mmol of free sulfate and 229.0 +/- 75.3 mumol of taurine/kg of body weight per day. When the diet was changed to a synthetic 25% casein diet, the taurine excretion decreased to 15% of the previous daily excretion, but sulfate excretion decreased only slightly. These decreased levels returned to the original levels when 5 mmol of L-cysteine/kg of body weight was administered into the stomach through a catheter. One week after the first L-cysteine administration, when sulfate and taurine excretion had returned to the original levels, 5 mmol of L-cysteine/kg of body weight was administered likewise. The rats excreted sulfur corresponding to about 95% of L-cysteine administered in the form of free sulfate and taurine within a few days following L-cysteine administration, and sulfate excretion was 3.5 times more than taurine excretion. These results seem to suggest that, in rats, sulfur metabolism is in a state of equilibrium and that sulfate is formed preferentially to taurine.

KEYWORDS: sulfate, taurine, cysteine, sulfur metabolism

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Sulfate and Taurine Excretion in Rats after L-Cysteine Administration

Shigeko Yoshida[a], Reiko Akagi[a] and Toshihiko Ubuka*

Department of Biochemistry, Okayama University Medical School, Okayama 700, Japan

Excretion of sulfate and taurine, two major metabolites of sulfur, was examined in rats to study the nutritional status of sulfur metabolism in the mammals. Rats maintained on a conventional laboratory diet excreted 1.83±0.14 mmol of free sulfate and 229.0±75.3 μmol of taurine/kg of body weight per day. When the diet was changed to a synthetic 25% casein diet, the taurine excretion decreased to 15% of the previous daily excretion, but sulfate excretion decreased only slightly. These decreased levels returned to the original levels when 5 mmol of L-cysteine/kg of body weight was administered into the stomach through a catheter. One week after the first L-cysteine administration, when sulfate and taurine excretion had returned to the original levels, 5 mmol of L-cysteine/kg of body weight was administered likewise. The rats excreted sulfur corresponding to about 95% of L-cysteine administered in the form of free sulfate and taurine within a few days following L-cysteine administration, and sulfate excretion was 3.5 times more than taurine excretion. These results seem to suggest that, in rats, sulfur metabolism is in a state of equilibrium and that sulfate is formed preferentially to taurine.

Key words : **sulfate, taurine, cysteine, sulfur metabolism**

Sulfate and taurine are two major end products of sulfur metabolism in mammals (1, 2). These metabolites play important roles in the animal body. They are involved in the biosynthesis of sulfated polysaccharides (3) and taurine conjugates of bile acids (4), detoxication reactions (5) and the function of the central nervous system and muscle (4).

Mammals take up sulfur mainly as methionion and cysteine in proteins (1). After digestion and absorption, these sulfur-containing amino acids enter the amino acid pool and are metabolized. Methionine is converted to cysteine by the transsulfuration reaction, and the sulfur of cysteine is ultimately oxidized to sulfate and taurine (1, 2), which are utilized in the body and finally excreted in the urine (6, 7). The present study was undertaken to examine the nutritional status of sulfur metabolism in mammals with special reference to sulfate and taurine, and urinary excretion of these metabolites was studied after cysteine administration to

a: Present address: Faculty of Food and Nutrition, Okayama Prefectural Junior College, 3-1-1 Ishimacho, Okayama 700, Japan

*To whom correspondence should be addressed.

rats fed a synthetic 25% casein diet.

Materials and Methods

Materials. Male Wistar rats weighing about 150 g were maintained on a laboratory diet, MF, of Oriental Yeast Co., Ltd., Tokyo, Japan, and water *ad libitum.* These rats were used in the present experiments after they had grown to 190-210 g of body weight.

Casein, corn starch(α), potato starch(α), cellulose powder, a mixture of vitamins, and a mixture of minerals were obtained from Oriental Yeast Co., Ltd. The synthetic diet (25% casein diet) was prepared shortly before use by mixing the above materials, vegetable oil and sucrose in the following proportion : casein, 25% ; corn starch, 38% ; potato starch, 10% ; cellulose powder, 8% ; mineral mixture (sulfate content, 3.6 mmol/100g dry diet, mostly as $MgSO_4$), 6% ; vitamin mixture, 2% ; vegetable oil, 6% ; sucrose, 5%. Growth curves of rats fed this synthetic diet was not significantly different from those of rats fed MF diet.

L-Cysteine was obtained from Sigma Chemical Co., St. Louis, MO, USA. Magnesium hydroxide carbonate was from Merck & Co., Darmstadt, F.R.G. Dowex 1 (×8, 200-400 mesh), Dowex 50 W (×8, 100-200 mesh and 200-400 mesh) and Econo columns (1.5×15 cm and 0.7×15 cm) were from Bio-Rad Laboratories, Richmond, CA, USA. Acetic acid, toluene, sodium hydroxide, barium perchlorate, trichloroacetic acid, ninhydrin and thorin [2-(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid] were of analytical grade and obtained from Wako Pure Chemical Ind., Osaka, Japan. Ethanol was used after distillation.

Feeding of rats and administration of L-cysteine. Nine rats were separated into two groups (A and B, n = 5 and 4, respectively). Each rat was housed in a metabolic cage and fed the 25% casein diet and water *ad libitvm.* Food intake and change of body weight were recorded daily. The 24-h urine was collected in a 100-ml Erlenmeyer flask containing 5 ml of 50% acetic acid and 5 ml of toluene. The urine was filtered and used for the determination of sulfate and taurine. One week after the change to the 25% casein diet, a freshly prepared L-cysteine solution (5 mmol/10 ml of water) was administered into the stomach through a catheter to rats of group A at 10 : 00 a.m. at a dose of 5 mmol/kg of body weight. Ten ml of water/kg of body weight was administered likewise to rats of group B as controls. Administration of L-cysteine solution and water was repeated 3 times at intervals of one week as shown in Figs. 3-5.

Determination of urinary sulfate by microtitration. Urinary free sulfate was determined by a modification of the method of Fritz and Yamamura which was applied to the determination of sulfate in water (8). Urine was adjusted to pH 7 with 2N NaOH. To 5.0 ml of the urine, 0.5 g of magnesium hydroxide carbonate was admixed. After 10 min the mixture was heated in a boiling water bath for 3 min and then chilled in an ice-water bath for 10 min. The magnesium phosphate formed was centrifuged off at 1200×*g* for 10 min. Three ml of the resulting supernatant was applied to a Dowex 50 W column (100-200 mesh, 1.5×10 cm, H^+ form), and the column was washed with water. The initial 30 ml of the effluent was collected and used for titration. To 5.0 ml of the effluent, 5.0 ml of 10% acetic acid and 40 ml of ethanol were added. The mixture was titrated with 5mM barium perchlorate using a microburette and a drop of 0.2% thorin as the indicator.

In some experiments, total (free+ethereal) sulfate was determined after hydrolysis with 0.2N hydrochloric acid (9) using 1.0 ml of rat urine. The amount of ethereal sulfate was obtained as the difference between total and free sulfate.

Determination of urinary sulfate by gas chromatography. Sulfate was determined also by the method of Masuoka *et al.* (10), and the results were compared with those obtained by the microtitration method described above. The gas chromatographic method was mainly used for the determination of total sulfate in the urine.

Determination of urinary taurine. Taurine was determined by ninhydrin reaction after the separation of the taurine fraction with a combination of anion-exchange and cation-exchange columns (11), as follows. Columns of Dowex 1 (200-400 mesh, acetate form, 0.7×10 cm, packed in a Econo column, 0.7×15 cm) and Dowex 50W (200-400 mesh, H^+ form, 0.5×6.5 cm, packed in a one-ml plastic syringe) were washed with water until the washings became neutral. Then the top

of the latter column was connected to the bottom of the former column using a short section of silicone rubber tubing with a minimum dead space. The rat urine was diluted 10 times with water, and 1.0 ml of the diluted urine was applied to the top of the combined column. The column was washed with 2.0 ml of water. The initial effluent and washing were discarded. Taurine was eluted with 5.0 ml of water. The pooled effluent (taurine fraction) was made up to 10.0 ml with water, and one ml was used for taurine determination using Moore's ninhydrin reagent (12). The resins used in the combined columns were regenerated separately.

High voltage paper electrophoresis was performed in pyridine(95%)-acetic acid-water (5 : 100 : 895, v/v, pH 3.1) (13) at 3 kV for 30 min. Amino acids were visualized with 1% ninhydrin-2% pyridine in acetone.

Statistical evaluation was performed by Student's t test.

Results and Discussion

Quantitative analysis of urinary sulfate by microtitration method. Fig. 1 is the standard curve of sulfate determined by the microtitration method. The curve was straight

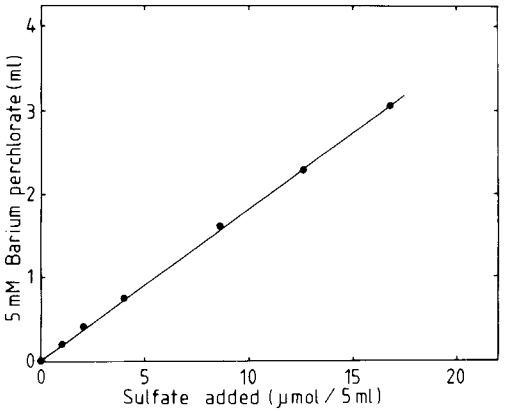

Fig. 1 Standard curve of sulfate determination by microtitration. Known amounts of potassium sulfate solution were added to the rat urine, which was treated as described under Materials and Methods and titrated with 5 mM barium perchlorate (f = 1.13) using 0.2% thorin as the indicator.

Table 1 Comparison of total urinary sulfate determined by microtitration and gas chromatography[a]

Sample no.	Sulfate determined (μmol/ml of urine)	
	Microtitration	Gas chromatography
1	26.66 ± 0.22	25.94 ± 1.15
2	36.37 ± 0.22	33.07 ± 0.10
3	7.91 ± 0.11	7.89 ± 0.48
4	6.33 ± 0.11	5.36 ± 0.40
5	17.63 ± 0.11	16.32 ± 0.99
6	37.06 ± 0.11	35.75 ± 1.16
7	16.95 ± 0.22	13.86 ± 0.62
8	16.72 ± 0.11	13.43 ± 0.80
9	16.50 ± 0.11	16.07 ± 0.60
10	13.45 ± 0.11	14.13 ± 0.80

a : Details of the analytical methods are described under Materials and Methods. Each value is the mean ± SD of 3 determinations.

when 12.5 to 200 μmol of sulfate was added to 5.0 ml of urine, and the recovery was 100.3 ± 2.9%.

Table 1 shows the comparison of the total sulfate determined by the microtitration and gas chromatographic methods. The values obtained by microtitration (y) agreed well with those obtained by gas chromatography (x). The regression line and the correlation coefficient were : $y = 1.038x + 0.68$, and $r = 0.992$. These results mean that the microtitration method can be used for the determination of urinary sulfate. The amount of ethereal sulfate in rat urines ranged from 4.4 to 12.9% of the total with an average ± SD of 8.88 ± 2.85% (n = 13).

Quantitative analysis of urinary taurine. Fig. 2 shows an elution profile of the rat urine from the combined ion-exchange column. High voltage paper electrophoresis revealed that the second peak was taurine and that it was chromatographically pure. The first small peak was cysteic acid. The large peak which was eluted after the taurine peak seemed to be ammonia, because the peak did not exhibit any ninhydrin-positive reaction after evaporation to dryness and

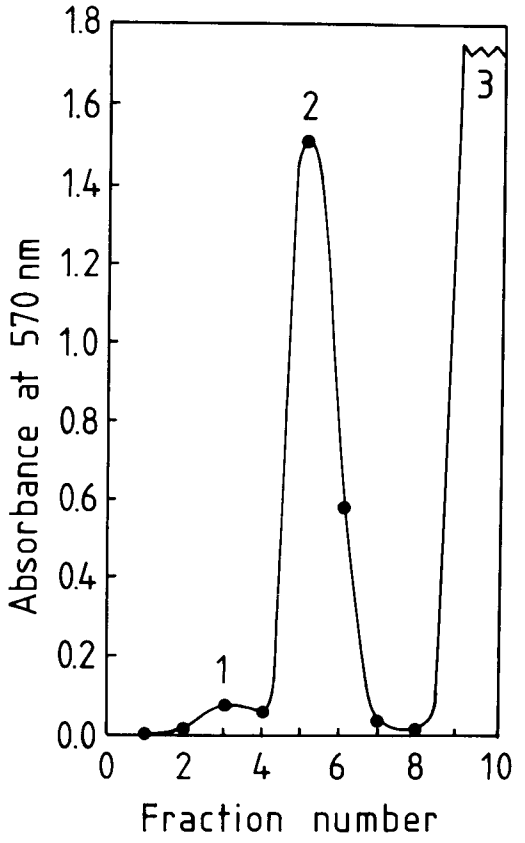

Fig. 2 Ion-exchange chromatography of rat urine with a combined column of Dowex 1 (acetate form, 0.7×10 cm) and Dowex 50 W (H^+ form, 0.5×6.5 cm). One ml of 10 : 1 diluted rat urine was applied and eluted with water, collecting one-ml fractions. Aliquots of the fractions were used for the ninhydrin reaction.

Table 2 Recovery of taurine determined by combined ion-exchange column chromatography [a]

Taurine added (μmol/ml)	Taurine determined (μmol/ml)	Difference (μmol/ml)	Recovery (%)
0.0	10.3±0.1		
5.0	15.2±0.1	4.9±0.1	97.2±2.0
10.0	20.1±0.1	9.8±0.1	97.8±1.2
20.0	29.4±0.2	19.5±0.2	97.6±0.8
50.0	60.5±0.3	50.1±0.3	100.2±0.7
90.0	100.6±0.3	90.3±0.4	100.3±0.4

a : Five to 90 μmol/ml of taurine was added to rat urine. Details of the procedure are described under Materials and Methods. Each value is the mean±SD of five determinations.

paper electrophoresis. As shown in Table 2, the recovery of taurine by the present method was over 97% when 5-90 μmol/ml of taurine was added to rat urines.

Methods for taurine determination using anion- and cation-exchange resins in combination have been reported (14-16). In these methods, two kinds of resins have been packed in one glass tube (14, 15) or used as two separate columns (16). The method used in the present study was preferred because the regeneration of resins was easy and the procedure was simple.

Growth of rats. Fig. 3 shows the growth curve of rats fed the synthetic diet. When rats were transferred from the conventional laboratory diet, MF, to the synthetic diet, the average body weight dropped significantly ($p < 0.001$) to about 90% because of the transient decrease in the food intake for a few days (data not shown). After the initial drop, the body weights returned gradually in two weeks to the constant growth curve, which was not different from that of rats fed MF without changing to the synthetic diet. Fig. 3 also shows that the body weights of rats which received L-cysteine did not differ significantly from those of control rats.

Excretion of free sulfate. Free sulfate excreted in the urine of rats fed the MF diet was 1.83±0.14 mmol/kg of body weight per day. Sulfate excretion after changing the diet from MF to the synthetic diet is shown in Fig 4. After the initial drop in body weight for the initial 3 days following the change in the diet, sulfate excretion per day per kg of body weight was relatively constant. It was very constant from 2 weeks after the change in the diet. The average excretion was 1.9±0.4 mmol/kg per day.

Fig. 4 also illustrates the sulfate excretion after L-cysteine administration to rats. When 5 mmol of L-cysteine per kg of body weight was administered to rats fed the synthetic diet for 7 days, about 4.0 mmol of

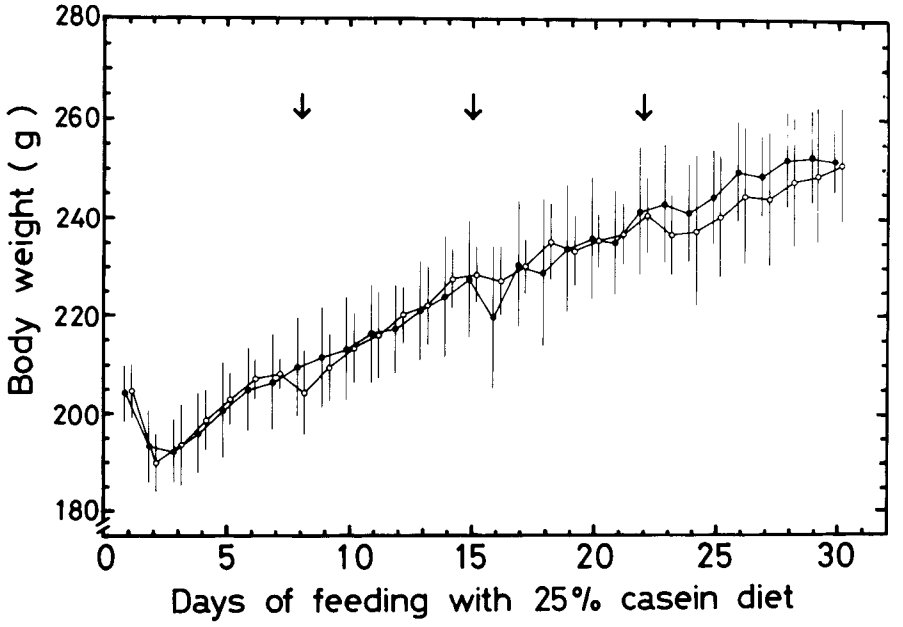

Fig. 3 Growth curves of rats fed a synthetic 25% casein diet. At the times indicated with arrows, 5 mmol of L-cysteine per kg of body weight was administered into the stomach through a catheter (●——●, n = 5). Control rats (○——○, n = 4) received no L-cysteine. Circles represent the mean values, and vertical lines represent standard deviation (SD).

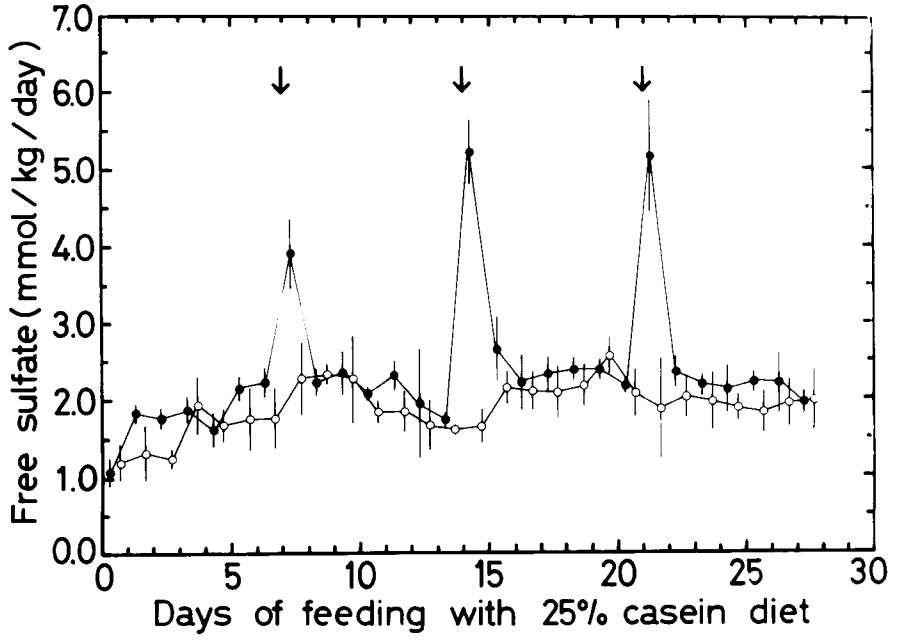

Fig. 4 Excretion of free sulfate in the urine of the same rats as shown in Fig. 3. Arrows indicate the times when 5 mmol of L-cysteine per kg of body weight was administered into the stomach through a catheter. For symbols, see the legend to Fig. 3.

free sulfate per kg of body weight was excreted in the urine collected for 24 h after the administration. Two weeks after the change in diet, when daily sulfate excretion was constant, L-cysteine was administered likewise. The average excretion of free sulfate in the next 24-h urine was 5.2 ± 0.4 mmol per kg of body weight. When L-cysteine was administered 3 weeks after the change of the diet, the excretion of free sulfate in the next 24-h urine was 5.2 ± 0.7 mmol per kg of body weight. Sulfate excretion within a few days of the peak excretion was slightly higher than that of the control rats. On the average, in addition to the daily excretion, 3.5 mmol of free sulfate per kg of body weight was excreted in the 24-h urine after the second and third L-cysteine administration, and 0.5 mmol per kg of free sulfate was excreted in the next few days. Thus, the sulfur corresponding to about 80% of the L-cysteine administered was excreted rapidly. These results agree with the previous data that 75-85% of the sulfur ingested as sulfur-containing amino acid residues in proteins is eventually excreted as free sulfate (17).

Excretion of taurine. Average taurine excretion in rats fed the MF diet was 229.0 ± 75.3 μmol/kg of body weight per day. Taurine excretion after the change in the diet is shown in Fig. 5. Taurine excretion per day per kg of body weight decreased significantly ($p < 0.001$) to 15% following the change in the diet from MF to the synthetic diet (Fig. 5). This low value continued for 3 weeks. When L-cysteine was administered to rats one week after the diet change, the taurine excretion returned to the original level of 0.2 mmol/kg of body weight per day, and this level was maintained. The second and third L-cysteine administration were performed to rats in this state. As shown in Fig. 5, the taurine excretion in

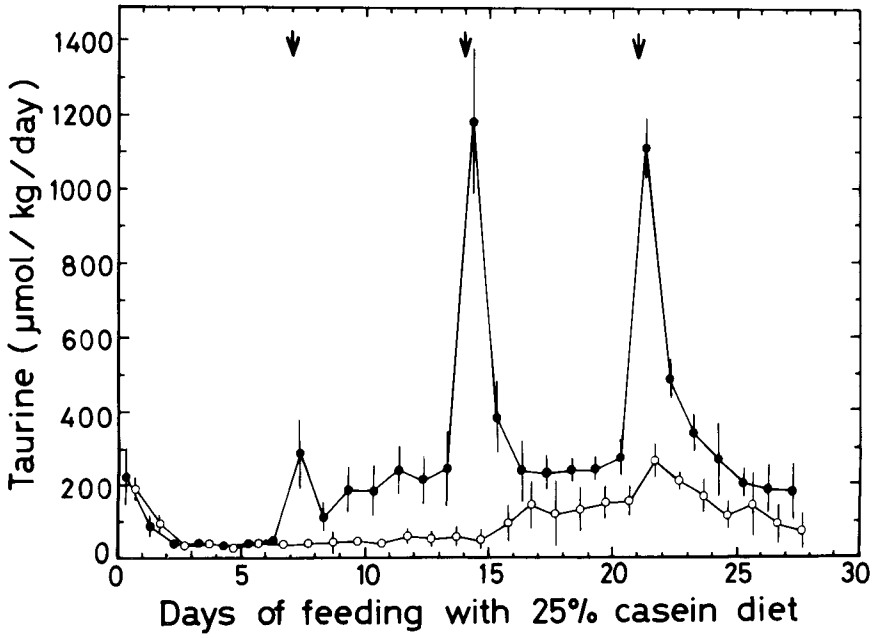

Fig. 5 Excretion of taurine in the urine of the same rats as shown in Figs. 3 and 4. Five mmol of L-cysteine per kg of body weight was administered at the times indicated with arrows. For symbols, see the legend to Fig. 3.

the next 24-h urine exhibited a sharp peak, amounting to 1.2 mmol per kg of body weight.

The present results show that the increase in the sum of the free sulfate and taurine excreted following L-cysteine administration amounts to over 95% of the L-cysteine administered. As reported previously (18), sulfur compounds in tissues of rats fed the same synthetic 25% casein diet and administered 5 mmol of L-cysteine per kg of body weight did not increase, indicating that L-cysteine was rapidly metabolized. These results seem to suggest that the sulfur metabolism in rats is in a state of equilibrium, which is similar to the well-known nitrogen balance.

Cysteine may be metabolized through several pathways : the pathway via cysteinesulfinate (oxidation pathway) and desulfuration pathways (19), including that via 3-mercaptopyruvate (transamination pathway) (20). The oxidation pathway is considered to be the major route of cysteine metabolism (2). It has been shown that considerable species variation exists between transamination and decarboxylation of cysteinesulfinate, the key reactions leading to the formation of sulfate and taurine, respectively (2). As shown in Figs. 4 and 5, the increase in sulfate excretion after L-cysteine administration was about 3.5 times more than that in taurine excretion. These results seem to suggest that sulfate formation in rats proceeds preferentially to taurine formation. As sulfate may be absorbed and excreted in urine(21), the decrease in taurine excretion in the present study might be an indication of insufficient intake of sulfur-containing amino acids.

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